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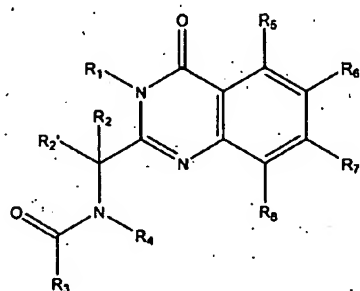
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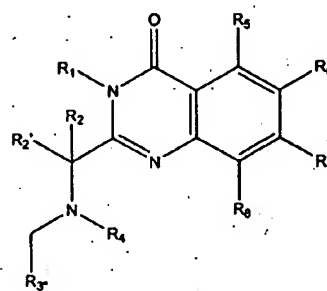
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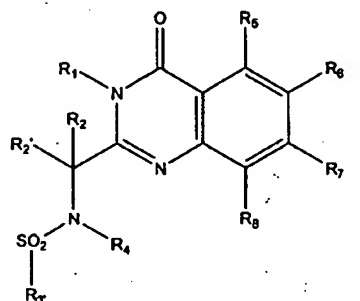
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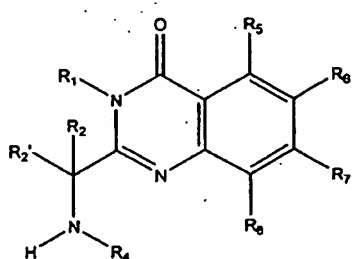
(a)



(b)



(c)



(d)

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(57) Abstract: Quinazolinones of formulae (a, b, c and d) are disclosed. They are useful for treating cellular proliferative diseases and disorders associated with KSP kinesin activity.

METHODS AND COMPOSITIONS UTILIZING QUINAZOLINONES

FIELD OF THE INVENTION

This invention relates to quinazolinone derivatives, which are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, for example cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders and inflammation.

BACKGROUND OF THE INVENTION

Interest in the medicinal chemistry of quinazoline derivatives was stimulated in the early 1950's with the elucidation of the structure of a quinazoline alkaloid, 3-[β -keto-gamma-(3-hydroxy-2-piperidyl)-propyl]-4-quinazolone, from an Asian plant known for its antimalarial properties. In a quest to find additional antimalarial agents, various substituted quinazolines have been synthesized. Of particular import was the synthesis of the derivative 2-methyl-3-o-tolyl-4-(3H)-quinazolinone. This compound, known by the name methaqualone, though ineffective against protozoa, was found to be a potent hypnotic.

Since the introduction of methaqualone and its discovery as a hypnotic, the pharmacological activity of quinazolinones and related compounds has been investigated. Quinazolinones and derivatives thereof are now known to have a wide variety of biological properties including hypnotic, sedative, analgesic, anticonvulsant, antitussive and anti-inflammatory activities.

Quinazolinone derivatives for which specific biological uses have been described include U.S. Patent No. 5,147,875 describing 2-(substituted phenyl)-4-oxo quinazolines with bronchodilator activity. U.S. Patent Nos. 3,723,432, 3,740,442, and 3,925,548 describe a class of 1-substituted-4-aryl-2(1H)-quinazolinone derivatives useful as anti-inflammatory agents. European patent publication EP 0 056 637 B1 claims a class of 4(3H)-quinazolinone derivatives for the treatment of hypertension. European patent publication EP 0 884 319 A1 describes pharmaceutical compositions of quinazolin-4-one derivatives used to treat neurodegenerative, psychotropic, and drug and alcohol induced central and peripheral nervous system disorders.

only the taxanes, but also the camptothecin class of topoisomerase I inhibitors. From both of these perspectives, mitotic kinesins are attractive targets for new anti-cancer agents.

Mitotic kinesins are enzymes essential for assembly and function of the mitotic spindle, but are not generally part of other microtubule structures, such as in nerve processes. Mitotic kinesins play essential roles during all phases of mitosis. These enzymes are "molecular motors" that transform energy released by hydrolysis of ATP into mechanical force which drives the directional movement of cellular cargoes along microtubules. The catalytic domain sufficient for this task is a compact structure of approximately 340 amino acids. During mitosis, kinesins organize microtubules into the bipolar structure that is the mitotic spindle. Kinesins mediate movement of chromosomes along spindle microtubules, as well as structural changes in the mitotic spindle associated with specific phases of mitosis. Experimental perturbation of mitotic kinesin function causes malformation or dysfunction of the mitotic spindle, frequently resulting in cell cycle arrest and cell death.

Among the mitotic kinesins that have been identified is KSP. KSP belongs to an evolutionarily conserved kinesin subfamily of plus end-directed microtubule motors that assemble into bipolar homotetramers consisting of antiparallel homodimers. During mitosis KSP associates with microtubules of the mitotic spindle. Microinjection of antibodies directed against KSP into human cells prevents spindle pole separation during prometaphase, giving rise to monopolar spindles and causing mitotic arrest and induction of programmed cell death. KSP and related kinesins in other, non-human, organisms, bundle antiparallel microtubules and slide them relative to one another, thus forcing the two spindle poles apart. KSP may also mediate in anaphase B spindle elongation and focussing of microtubules at the spindle pole.

Human KSP (also termed HsEg5) has been described [Blangy, et al., Cell, 83:1159-69 (1995); Whitehead, et al., Arthritis Rheum., 39:1635-42 (1996); Galgio et al., J. Cell Biol., 135:339-414 (1996); Blangy, et al., J Biol. Chem., 272:19418-24 (1997); Blangy, et al., Cell Motil Cytoskeleton, 40:174-82 (1998); Whitehead and Rattner, J. Cell Sci., 111:2551-61 (1998); Kaiser, et al., JBC 274:18925-31 (1999); GenBank accession numbers: X85137, NM004523 and U37426], and a fragment of the KSP

wherein:

R₁ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

5 R₂ and R₂' are independently chosen from hydrogen, alkyl, oxaalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; or R₂ and R₂' taken together form a 3- to 7-membered ring;

10 R₃ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, oxaalkyl, oxaalkylaryl, substituted oxaalkylaryl, oxaalkyl heteroaryl, substituted oxaalkylheteroaryl, R₁₅O- and R₁₅-NH-;

15 R₃' is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl and R₁₅-NH-;

R₃'' is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

20 R₄ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and R₁₆-alkylene-;

25 R₅, R₆, R₇ and R₈ are independently chosen from hydrogen, alkyl, alkoxy, halogen, fluoroalkyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, sulfonamidoalkyl, sulfonamidoaryl, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, aryl and heteroaryl;

R₁₅ is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

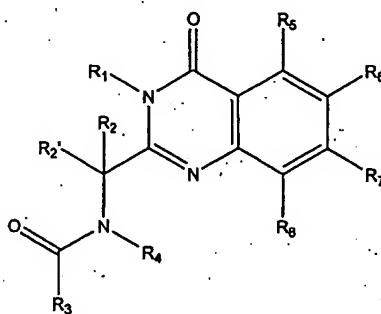
30 R₁₆ is chosen from alkoxy, amino, alkylamino, dialkylamino, N-heterocyclyl and substituted N-heterocyclyl.

Diseases and disorders that respond to therapy with compounds of the invention include cancer, hyperplasia, restenosis, cardiac hypertrophy, immune disorders and

capitalizes on the finding that perturbation of mitotic kinesin function causes malformation or dysfunction of mitotic spindles, frequently resulting in cell cycle arrest and cell death. The methods of inhibiting a human KSP kinesin comprise contacting an inhibitor of the invention with a KSP kinesin, particularly human KSP kinesins, including fragments and variants of KSP. The inhibition can be of the ATP hydrolysis activity of the KSP kinesin and/or the mitotic spindle formation activity, such that the mitotic spindles are disrupted. Meiotic spindles may also be disrupted.

An object of the present invention is to develop inhibitors and modulators of mitotic kinesins, in particular KSP, for the treatment of disorders associated with cell proliferation. Traditionally, dramatic improvements in the treatment of cancer, one type of cell proliferative disorder, have been associated with identification of therapeutic agents acting through novel mechanisms. Examples of this include not only the taxane class of agents that appear to act on microtubule formation, but also the camptothecin class of topoisomerase I inhibitors. The compositions and methods described herein can differ in their selectivity and are preferably used to treat diseases of proliferating cells, including, but not limited to cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders and inflammation.

Accordingly, the present invention relates to methods employing quinazolinone amides of formula 1a:



1a

20

- R_3 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, oxaalkyl, oxaalkylaryl, substituted oxaalkylaryl, oxaalkylheteroaryl, substituted oxaalkylheteroaryl, $R_{15}O-$ and $R_{15}NH-$;
- 5 R_3 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and $R_{15}NH-$;
- R_3 is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted
- 10 alkylheteroaryl;
- R_4 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and R_{16} -alkylene-;
- R_5 , R_6 , R_7 and R_8 are independently chosen from hydrogen, alkyl, alkoxy, halogen,
- 15 fluoroalkyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, sulfonamidoalkyl, sulfonamidoaryl, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, aryl and heteroaryl;
- R_{15} is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted
- 20 alkylheteroaryl;
- R_{16} is chosen from alkoxy, amino, alkylamino, dialkylamino, N-heterocyclyl and substituted N-heterocyclyl.

All of the compounds falling within the foregoing parent genus and its subgenera are useful as kinesin inhibitors, but not all the compounds are novel. In particular, certain

25 ureas (i.e. compounds in which R_3 is $R_{15}NH$) are disclosed in US patent 5,756,502 as agents which modify cholecystokinin action. The specific exceptions in the claims reflect applicants' intent to avoid claiming subject matter that, while functionally part of the inventive concept, is not patentable to them for reasons having nothing to do with the scope of the invention.

cyclopropyloxy, cyclohexyloxy and the like. Lower-alkoxy refers to groups containing one to four carbons.

Acyl refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated and aromatic and combinations thereof, attached to the parent structure through a carbonyl functionality. One or more carbons in the acyl residue may be replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, benzyloxycarbonyl and the like. Lower-acyl refers to groups containing one to four carbons.

Aryl and heteroaryl mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0-3 heteroatoms selected from O, N, or S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S; or a tricyclic 13- or 14-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S. The aromatic 6- to 14-membered carbocyclic rings include, e.g., benzene, naphthalene, indane, tetralin, and fluorene and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole and pyrazole.

Alkylaryl refers to a residue in which an aryl moiety is attached to the parent structure via an alkylene residue. Examples are benzyl, phenethyl, phenylvinyl, phenylallyl and the like. Oxaalkyl and oxaalkylaryl refer to alkyl and alkylaryl residues in which one or more methylenes have been replaced by oxygen. Examples of oxaalkyl and oxaalkylaryl residues are ethoxyethoxyethyl (3,6-dioxaoctyl), benzyloxymethyl and phenoxymethyl; in general, glycol ethers, such as polyethyleneglycol, are intended to be encompassed by this group. Alkylheteroaryl refers to a residue in which a heteroaryl moiety is attached to the parent structure via an alkylene residue. Examples include furanylmethyl, pyridinylmethyl, pyrimidinylethyl and the like.

Heterocycle means a cycloalkyl or aryl residue in which one to four of the carbons is replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples of heterocycles that fall within the scope of the invention include imidazoline,

substituted with a plurality of halogens, but not necessarily a plurality of the same halogen; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl.

Most of the compounds described herein contain one or more asymmetric centers (e.g. the carbon to which R_2 and R_2' are attached) and may thus give rise to enantiomers, 5 diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When 10 the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included.

When desired, the R- and S-isomers may be resolved by methods known to those 15 skilled in the art, for example by formation of diastereoisomeric salts or complexes which may be separated, for example, by crystallisation; via formation of diastereoisomeric derivatives which may be separated, for example, by crystallisation, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed 20 by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step may be 25 required to liberate the desired enantiomeric form. Alternatively, specific enantiomer may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation. An example of a synthesis from optically active starting materials is shown in Figure 4.

30 In one embodiment, as will be appreciated by those in the art, the two adjacent R_2 groups may be fused together to form a ring structure. Again, the fused ring structure

In a preferred embodiment R_3 is selected from chosen from alkyl, substituted alkyl, alkylaryl, heteroaryl, aryl, substituted aryl, substituted oxaalkylaryl, $-O-R_{15}$ and $-NH-R_{15}$, and R_{15} is chosen from alkyl, aryl and substituted aryl.

5 In a more preferred embodiment, when R_3 is not $-NHR_{15}$, R_3 is chosen from C_1-C_{13} alkyl; substituted lower alkyl; aryl, including phenyl, biphenyl and naphthyl; substituted aryl, including phenyl substituted with one or more halo, lower alkyl, loweralkoxy, nitro, carboxy, methylenedioxy or trifluoromethyl; benzyl; phenoxymethyl; halophenoxymethyl; phenylvinyl; heteroaryl; heteroaryl substituted with lower alkyl; and benzyloxymethyl.

10 In a most preferred embodiment, when R_3 is not $-NHR_{15}$, R_3 is chosen from ethyl, propyl, chloropropyl, butoxy, heptyl, butyl, octyl, tridecanyl, (ethoxycarbonyl)ethyl, dimethylaminoethyl, dimethylaminomethyl, phenyl, naphthyl, halophenyl, dihalophenyl, cyanophenyl, halo(trifluoromethyl)phenyl, chlorophenoxymethyl, methoxyphenyl, carboxyphenyl, ethylphenyl, tolyl, biphenyl, methylenedioxyphenyl, 15 methylsulfonylphenyl, methoxychlorophenyl, chloronaphthyl, methylhalophenyl, trifluoromethylphenyl, butylphenyl, pentylphenyl, methylnitrophenyl, phenoxymethyl, dimethoxyphenyl, phenylvinyl, nitrochlorophenyl, nitrophenyl, dinitrophenyl, bis(trifluoromethyl)phenyl, benzyloxymethyl, benzyl, furanyl, benzofuranyl, pyridinyl, indolyl, methylpyridinyl, quinolinyl, picolinyl, pyrazolyl, and imidazolyl.

20 In a more preferred embodiment, when R_3 is $-NHR_{15}$, R_{15} is chosen from lower alkyl; cyclohexyl; phenyl; and phenyl substituted with halo, lower alkyl, loweralkoxy, or lower alkylthio.

In a most preferred embodiment, when R_3 is $-NHR_{15}$, R_{15} is isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, 25 trifluoromethylphenyl or methylthiophenyl.

In a preferred embodiment R_4 is chosen from alkyl, aryl, alkylaryl, alkylheteroaryl, substituted alkyl, substituted aryl, and $-alkylene-R_{16}$, and R_{16} is chosen from alkoxy, amino, alkylamino, dialkylamino and N-heterocycl.

amino, propylamino or azetidiny; R₅ is hydrogen; R₆ is hydrogen; R₇ is halo; and R₈ is hydrogen.

When considering primarily the sulfonamides of formula 1b, R₁ is preferably chosen from hydrogen, lower alkyl, substituted lower alkyl, benzyl, substituted benzyl, phenyl and substituted phenyl; R₂ is chosen from hydrogen and lower alkyl and R₂' is hydrogen; R₃ is chosen from C₁-C₁₃ alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, nitro, methylenedioxy, or trifluoromethyl; biphenyl and heteroaryl; and R₄ is chosen from lower alkyl, cyclohexyl; phenyl substituted with hydroxy, lower alkoxy or lower alkyl; benzyl; heteroarylmethyl; heteroarylethyl; heteroarylpropyl; heteroarylethyl; heteroarylpropyl and -alkylene-R₁₆, wherein R₁₆ is di(lower alkyl)amino, (lower alkyl)amino, amino, lower alkoxy, or N-heterocyclyl, particularly pyrrolidino, piperidino or imidazolyl.

When considering primarily the sulfonamides of formula 1b, R₁ is most preferably chosen from lower alkyl, benzyl, substituted benzyl and substituted phenyl; R₂ is hydrogen or lower alkyl; R₂' is hydrogen; R₃ is chosen from substituted phenyl and naphthyl; R₄ is -alkylene-R₁₆; R₇ is hydrogen, fluoro, methyl or chloro; R₅, R₆ and R₈ are hydrogen; and R₁₆ is chosen from di(lower alkylamino), (lower alkyl)amino, amino, pyrrolidino, piperidino, imidazolyl and morpholino.

When considering primarily the amines of formulae 1c and 1d, R₁ is preferably chosen from hydrogen, lower alkyl, substituted lower alkyl, benzyl, substituted benzyl, phenyl, naphthyl and substituted phenyl; R₂ is chosen from hydrogen, lower alkyl and substituted lower alkyl and R₂' is hydrogen; R₃ is chosen from C₁-C₁₃ alkyl; substituted lower alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, nitro, methylenedioxy, or trifluoromethyl; biphenyl, benzyl and heterocyclyl; and R₄ is chosen from lower alkyl; cyclohexyl; phenyl substituted with hydroxy, lower alkoxy or lower alkyl; benzyl; substituted benzyl; heterocyclyl; heteroarylmethyl; heteroarylethyl; heteroarylpropyl and -alkylene-R₁₆, wherein R₁₆ is di(lower alkyl)amino, (lower alkyl)amino, amino, lower alkoxy, or N-heterocyclyl.

When considering primarily the amines of formulae 1c and 1d, R₁ is most preferably chosen from lower alkyl, benzyl, substituted benzyl and substituted phenyl; R₂ is

- 5 d. In the case of formulae 1a and 1d, especially those where R_3 or $R_{3'}$ is aryl (preferably phenyl), substituted aryl (preferably lower alkyl- or lower alkoxy-substituted phenyl), alkylaryl (preferably benzyl and phenylvinyl), alkylheteroaryl, oxaalkylaryl (preferably phenoxy lower alkyl), oxaalkylheteroaryl, substituted alkylaryl (preferably substituted benzyl and substituted phenylvinyl), substituted alkylheteroaryl, substituted oxaalkylaryl (preferably substituted phenoxy lower alkyl), or substituted oxaalkylheteroaryl.
- 10 i. Most preferably those where R_3 or $R_{3'}$ is aryl, substituted aryl, lower alkylaryl or substituted lower alkylaryl.
2. Any of formulae 1a, 1b, 1c or 1d, where the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration, particularly where $R_{2'}$ is hydrogen:
- a. Especially where R_2 is lower alkyl (preferably ethyl, i-propyl, c-propyl or t-butyl).
- 15 i. Most preferably where R_2 is i-propyl.
- b. Especially where R_4 is substituted alkyl (preferably a primary-, secondary- or tertiary-amino-substituted lower alkyl).
- i. Most preferably where R_4 is primary amino-lower alkyl.
- c. Especially where R_5 , R_6 , and R_8 are hydrogen.
- 20 i. Most preferably where R_7 is hydrogen, halo (particularly chloro or fluoro), lower alkyl (particularly methyl), substituted lower alkyl, lower alkoxy (particularly methoxy), or cyano
1. Especially where R_7 is chloro.
3. Any of formulae 1a, 1b, 1c or 1d where R_7 is hydrogen, halo (preferably chloro or fluoro), lower alkyl (preferably methyl), substituted lower alkyl, lower alkoxy (preferably methoxy), or cyano.
- 25 a. Especially those where R_7 is halo or cyano.
- b. Especially those where R_5 , R_6 , and R_8 are hydrogen.
- i. Most preferably those where R_7 is chloro.

- i. Particularly those where R_3 is aryl, substituted aryl, lower alkylaryl, substituted lower alkylaryl, oxa(lower)alkylaryl.
 - 1. Most preferably those where R_3 is methyl- and/or halo-substituted phenyl.
- 5 c. Especially those where R_4 is substituted alkyl (preferably a primary-, secondary- or tertiary-amino-substituted lower alkyl).
 - i. Particularly those where R_4 is a primary-amino-substituted lower alkyl.
 - 1. Most preferably those where R_4 is 3-amino-n-propyl.
- 10 d. Especially those where R_5 , R_6 , R_7 and R_8 are chosen from hydrogen, halo (preferably chloro and fluoro), lower alkyl (preferably methyl), substituted lower alkyl, lower alkoxy (preferably methoxy), and cyano.
 - i. Particularly those where R_5 , R_6 , R_7 and R_8 are hydrogen, halo, lower alkyl or cyano.
 - 15 1. Most preferably those where R_5 , R_6 and R_8 are hydrogen.
 - a. Especially those where R_7 is halo or cyano (most preferably chloro).
 - 2. Especially those where R_7 is halo or cyano (most preferably chloro).
- 20 2. Where the stereogenic center to which R_2 and R_2' are attached is of the R configuration (preferably where R_2' is hydrogen).
 - a. Especially those where R_2 is lower alkyl (preferably ethyl, i-propyl, c-propyl or t-butyl).
 - i. Most preferably those where R_2 is i-propyl.
- 25 b. Especially those where R_3 is aryl (preferably phenyl), substituted aryl (preferably lower alkyl-, lower alkoxy-, and/or halo-substituted phenyl), alkylaryl (preferably benzyl and phenylvinyl), alkylheteroaryl, oxaalkylaryl (preferably phenoxy lower alkyl), oxaalkylheteroaryl, substituted alkylaryl (preferably substituted benzyl and substituted phenylvinyl), substituted
- 30 alkylheteroaryl, substituted oxaalkylaryl (preferably substituted phenoxy lower alkyl), or substituted oxaalkylheteroaryl.

- i. Particularly those where R_4 is a primary-amino-substituted lower alkyl.
 - 1. Most preferably those where R_4 is 3-amino-n-propyl.
- c. Especially those where R_5 , R_6 , R_7 and R_8 are chosen from hydrogen, halo (preferably chloro and fluoro), lower alkyl (preferably methyl), substituted lower alkyl, lower alkoxy (preferably methoxy), and cyano.
 - i. Particularly those where R_5 , R_6 , R_7 and R_8 are hydrogen, halo, lower alkyl or cyano.
 - 1. Most preferably those where R_5 , R_6 and R_8 are hydrogen.
 - a. Especially those where R_7 is halo or cyano (most preferably chloro).
 - 2. Especially those where R_7 is halo or cyano (most preferably chloro).
- 4. R_4 is substituted alkyl (preferably a primary-, secondary- or tertiary-amino-substituted lower alkyl).
 - a. Particularly those where R_4 is a primary-amino-substituted lower alkyl.
 - i. Most preferably those where R_4 is 3-amino-n-propyl.
 - b. Especially those where R_5 , R_6 , R_7 and R_8 are chosen from hydrogen, halo (preferably chloro and fluoro), lower alkyl (preferably methyl), substituted lower alkyl, lower alkoxy (preferably methoxy), and cyano.
 - i. Particularly those where R_5 , R_6 , R_7 and R_8 are hydrogen, halo, lower alkyl or cyano.
 - 1. Most preferably those where R_5 , R_6 and R_8 are hydrogen.
 - a. Especially those where R_7 is halo or cyano (most preferably chloro).
 - 2. Especially those where R_7 is halo or cyano (most preferably chloro).
- 5. R_5 , R_6 , R_7 and R_8 are chosen from hydrogen, halo (preferably chloro and fluoro), lower alkyl (preferably methyl), substituted lower alkyl, lower alkoxy (preferably methoxy), and cyano.

disrupted) by disturbing equilibrium, either by inhibiting or activating certain components. Similar approaches may be used to alter meiosis.

In a preferred embodiment, the compositions of the invention are used to modulate mitotic spindle formation, thus causing prolonged cell cycle arrest in mitosis. By
5 "modulate" herein is meant altering mitotic spindle formation, including increasing and decreasing spindle formation. By "mitotic spindle formation" herein is meant organization of microtubules into bipolar structures by mitotic kinesins. By "mitotic spindle dysfunction" herein is meant mitotic arrest and monopolar spindle formation.

The compositions of the invention are useful to bind to and/or modulate the activity of
10 a mitotic kinesin, KSP. In a preferred embodiment, the KSP is human KSP, although KSP kinesins from other organisms may also be used. In this context, modulate means either increasing or decreasing spindle pole separation, causing malformation, i.e., splaying, of mitotic spindle poles, or otherwise causing morphological perturbation of the mitotic spindle. Also included within the definition of KSP for
15 these purposes are variants and/or fragments of KSP. See U.S. Patent Application "Methods of Screening for Modulators of Cell Proliferation and Methods of Diagnosing Cell Proliferation States", filed Oct. 27, 1999 (U.S. Serial Number 09/428,156), hereby incorporated by reference in its entirety. In addition, other mitotic kinesins may be used in the present invention. However, the compositions of
20 the invention have been shown to have specificity for KSP.

For assay of activity, generally either KSP or a compound according to the invention is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble support may be made of any composition to which the compositions can be bound, is readily separated from
25 soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, Teflon™, etc. Microtiter plates and arrays
30 are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner

PCA. Phosphate standards are used so data can be converted to mM inorganic phosphate released. When all reactions and standards have been quenched in PCA, 100 μ L of malachite green reagent is added to the relevant wells in e.g., a microtiter plate. The mixture is developed for 10-15 minutes and the plate is read at an
5 absorbance of 650 nm. If phosphate standards were used, absorbance readings can be converted to mM P_i and plotted over time. Additionally, ATPase assays known in the art include the luciferase assay.

ATPase activity of kinesin motor domains also can be used to monitor the effects of modulating agents. In one embodiment ATPase assays of kinesin are performed in the
10 absence of microtubules. In another embodiment, the ATPase assays are performed in the presence of microtubules. Different types of modulating agents can be detected in the above assays. In a preferred embodiment, the effect of a modulating agent is independent of the concentration of microtubules and ATP. In another embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing the
15 concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the modulating agent is increased by increasing concentrations of ATP, microtubules or both.

Agents that modulate the biochemical activity of KSP in vitro may then be screened in vivo. Methods for such agents in vivo include assays of cell cycle distribution, cell
20 viability, or the presence, morphology, activity, distribution, or amount of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent Application "Methods of Screening for Modulators of Cell Proliferation and Methods of Diagnosing Cell
25 Proliferation States," filed Oct. 22, 1999, serial number 09/428,156, hereby incorporated by reference in its entirety.

In addition to the assays described above, microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J. Cell
30 biol., 135:399-414).

Another measure of inhibition is GI_{50} , defined as the concentration of the compound that results in a decrease in the rate of cell growth by fifty percent. Preferred compounds have GI_{50} 's of less than about 1 mM. The level of preferability of embodiments is a function of their GI_{50} : those having GI_{50} 's of less than about 20 μ M are more preferred; those having GI_{50} 's of 10 μ M more so; those having GI_{50} of less than about 1 μ M more so; those having GI_{50} 's of 100 nM more so; those having GI_{50} of less than about 10 nM even more so. Measurement of GI_{50} is done using a cell proliferation assay.

The compositions of the invention are used to treat cellular proliferation diseases. Disease states which can be treated by the methods and compositions provided herein include, but are not limited to, cancer (further discussed below), autoimmune disease, arthritis, graft rejection, inflammatory bowel disease, proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like. It is appreciated that in some cases the cells may not be in a hyper or hypo proliferation state (abnormal state) and still require treatment. For example, during wound healing, the cells may be proliferating "normally", but proliferation enhancement may be desired. Similarly, as discussed above, in the agriculture arena, cells may be in a "normal" state, but proliferation modulation may be desired to enhance a crop by directly enhancing growth of a crop, or by inhibiting the growth of a plant or organism which adversely affects the crop. Thus, in one embodiment, the invention herein includes application to cells or individuals afflicted or impending affliction with any one of these disorders or states.

The compositions and methods provided herein are particularly deemed useful for the treatment of cancer including solid tumors such as skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that may be treated by the compositions and methods of the invention include, but are not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus

melanoma, basal cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above-identified conditions.

- 5 Accordingly, the compositions of the invention are administered to cells. By "administered" herein is meant administration of a therapeutically effective dose of the mitotic agents of the invention to a cell either in cell culture or in a patient. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. By "cells" herein is meant almost any cell in which mitosis or meiosis can be altered.

A "patient" for the purposes of the present invention includes both humans and other animals, particularly mammals, and other organisms. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, and in the most preferred embodiment the patient is human.

- 20 Mitotic agents having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a patient, as described herein. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt.%. The agents may be administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents.

- In a preferred embodiment, the pharmaceutical compositions are in a water soluble form, such as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not

intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the anti-mitotic agents may be directly applied as a solution or spray.

To employ the compounds of the invention in a method of screening for compounds
5 that bind to KSP kinesin, the KSP is bound to a support, and a compound of the invention (which is a mitotic agent) is added to the assay. Alternatively, the compound of the invention is bound to the support and KSP is added. Classes of compounds among which novel binding agents may be sought include specific
10 antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc.. Of particular interest are screening assays for candidate agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays
(phosphorylation assays, etc.) and the like.

15 The determination of the binding of the mitotic agent to KSP may be done in a number of ways. In a preferred embodiment, the mitotic agent (the compound of the invention) is labeled, for example, with a fluorescent or radioactive moiety and binding determined directly. For example, this may be done by attaching all or a
20 portion of KSP to a solid support, adding a labeled mitotic agent (for example a compound of the invention in which at least one atom has been replaced by a detectable isotope), washing off excess reagent, and determining whether the amount of the label is that present on the solid support. Various blocking and washing steps may be utilized as is known in the art.

By "labeled" herein is meant that the compound is either directly or indirectly labeled
25 with a label which provides a detectable signal, e.g., radioisotope, fluorescent tag, enzyme, antibodies, particles such as magnetic particles, chemiluminescent tag, or specific binding molecules, etc. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule
30 which provides for detection, in accordance with known procedures, as outlined above. The label can directly or indirectly provide a detectable signal.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

Competitive screening assays may be done by combining KSP and a drug candidate in a first sample. A second sample comprises a mitotic agent, KSP and a drug candidate. This may be performed in either the presence or absence of microtubules. The binding of the drug candidate is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of binding to KSP and potentially modulating its activity. That is, if the binding of the drug candidate is different in the second sample relative to the first sample, the drug candidate is capable of binding to KSP.

In a preferred embodiment, the binding of the candidate agent is determined through the use of competitive binding assays. In this embodiment, the competitor is a binding moiety known to bind to KSP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding as between the candidate agent and the binding moiety, with the binding moiety displacing the candidate agent.

In one embodiment, the candidate agent is labeled. Either the candidate agent, or the competitor, or both, is added first to KSP for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C.

Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high throughput screening. Typically between 0.1 and 1 hour will be

Positive controls and negative controls may be used in the assays. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are incorporated by reference in their entirety.

EXAMPLES

Abbreviations and Definitions

The following abbreviations and terms have the indicated meanings throughout:

Ac	=	acetyl
BNB	=	4-bromomethyl-3-nitrobenzoic acid
Boc	=	t-butyloxy carbonyl
Bu	=	butyl
c-	=	cyclo
CBZ	=	carbobenzyloxy = benzyloxycarbonyl
DBU	=	diazabicyclo[5.4.0]undec-7-ene
DCM	=	dichloromethane = methylene chloride = CH_2Cl_2
DCE	=	dichloroethylene
DEAD	=	diethyl azodicarboxylate
DIC	=	diisopropylcarbodiimide

dropping funnel, and an efficient magnetic stir bar, was added anthranilic acid (1) (0.5 mole, 68.5 g) and dimethyl formamide (250 mL). To this solution was added butyryl chloride (0.55 mole, 57.1 mL) dropwise at such a rate that the temperature of the mixture did not rise above 40°C. The suspension was stirred vigorously at room temperature for at least an additional 3 h. The mixture was poured into water (2000 mL) and stirred for another 1 h. The precipitated product was collected by filtration, washed with cold water, and dried under reduced pressure over P₂O₅, yielding compound 2 (67.3 g, 65%).

Step 2: 2-Propyl-3,1-[4H]benzoxazin-4-one.

Compound 2 (51.8 g, 0.25 mole) was dissolved in acetic anhydride (180 mL) in a 500 mL round-bottom flask equipped with a magnetic stir bar, a Claisen-distillation head (with vacuum inlet) and a thermometer. The flask was placed in an oil bath and slowly heated to 170-180°C with vigorous stirring. The acetic acid produced was slowly distilled off under atmospheric pressure. Monitoring the head temperature of the distillation unit was used to follow the progress of the transformation. The reaction mixture was then cooled to 60 °C and the excess of acetic anhydride removed by distillation under reduced pressure (ca. 20 mm Hg). The residue was afterward cooled and the product crystallized. The product was triturated with n-hexane (75 mL) and isolated by filtration to yield 2-propyl-3,1-[4H]benzoxazin-4-one (3) (29.3 g, 62%). The above procedure gave compound 3 sufficiently pure to use directly in the next step.

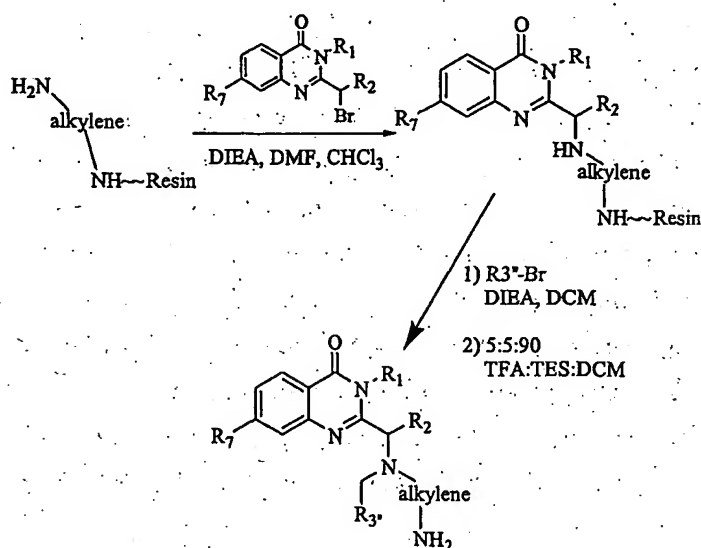
Step 3: 2-Propyl-3-benzylquinazolin-4-one.

Compound 3 (28.4 g, 0.15 mole) and benzylamine (17.5 mL, 0.16 mole) were refluxed in chloroform (50 mL) in a one-neck 250 mL round-bottom flask for 6 h. After complete consumption of compound 3, the chloroform was evaporated under reduced pressure. Ethylene glycol (100 mL) and NaOH pellets (0.60 g) were added to the residue and the flask equipped with a Claisen-distillation head and a magnetic stir bar. The flask was immersed in an oil bath and reheated to 130-140 °C bath temperature with vigorous stirring and maintained there for 5 h while the water produced was removed by distillation. After completion of the reaction, the clear solution was allowed to cool to room temperature and kept overnight to precipitate the

vessel using a Beckman Biomet 2000 automated liquid dispenser. The reaction mixture was shaken using a mechanical shaker, sonicated in an ultrasonic water bath, and then incubated overnight at room temperature. The mixture was diluted in CHCl_3 (300 μL) and washed with 5% aqueous NaHCO_3 and water. The solvent was removed in vacuo to provide compound 6 (65%). The purity of the compound was analyzed by TLC eluted with CH_2Cl_2 -ethanol-concentrated aqueous NH_3 , 100:10:1.

Examples 2 and 3

Synthesis of compounds of General Formula 1d



All anhydrous solvents were purchased from Aldrich chemical company in SureSeal® containers. Most reagents were purchase from Aldrich Chemical Company. Abbreviations: DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; TES, triethylsilane; TFA, trifluoroacetic acid. Array synthesis was conducted in 15 x 75 mm glass round bottom screw-cap vials contained in a 4 x 6 array aluminum synthesis block, sealed with a Teflon-lined rubber membrane. Reagents were added and aqueous extractions performed with single or multichannel pipettors. Filtrations were performed using Whatman/Polyfritronics 24 well, 10 mL filtration blocks. Evaporation of volatile materials from the array was performed with a Labconco Vortex-Evaporator or by sweeping with a 4 x 6 nitrogen manifold.

STEP 2: The quinazolinone ethyl-diamine resin (105 mg, 0.10 mmol) was placed into each of the vials in the first 2 rows of the array, and the quinazolinone propyl-diamine resin (88 mg, 0.10 mmol) was placed into each vial of the last 2 rows of the array. To each vial was added DIEA (0.131 mL, 0.75 mmol). Into each vial of the first 2 rows

5 of the array was added a different amine, and the additions were repeated for the last two rows of the array. The reaction block was shaken at 70 °C overnight. Liquid was removed from each vial by multichannel pipette using fine-pointed gel-well tips, and the resins were washed (2 x DCM, 1 x MeOH, 1 x DCM) and dried under vacuum.

STEP 3: To each vial of the array was added 2 mL of a 10:5:85 TFA:TES:DCM

10 solution. The reaction block was shaken for 45 min and the mixtures were transferred to a filter block, filtered, and washed twice with 0.75 mL DCM. The solutions were evaporated to yield yellow-to-red oils. These thick oils were triturated twice with ether, dissolved in DCM and treated with 4 M HCl in dioxane to provide the HCl salts (unknown number of salts per compound) as tan-to-white powdery or amorphous

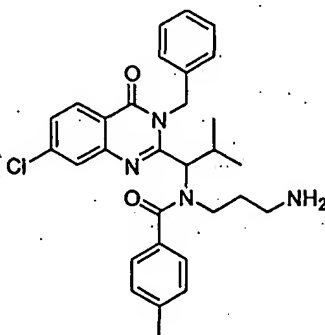
15 solids. Analysis by LCMS showed all to be >75 % pure.

Examples 4-6

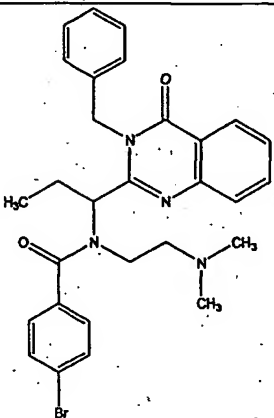
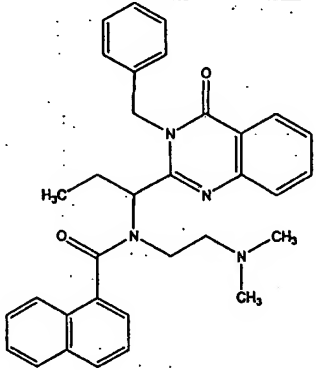
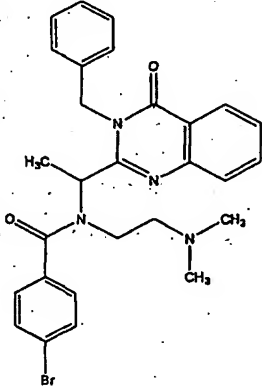
Six racemic quinazolinones were separated into their enantiomers by chiral chromatography. The chiral chromatography of three of these compounds is

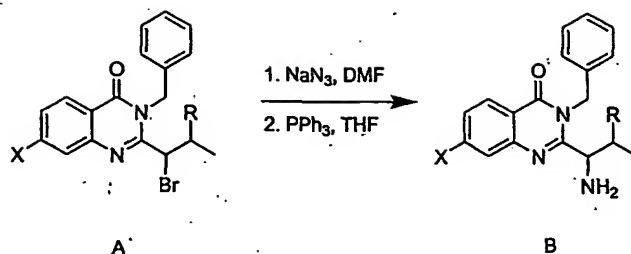
20 described below:

Example 4



25 Column - Chiralpak AD, 250 x 4.6 mm (Diacel Inc.). Sample - 0.5 mg/mL in EtOH.

	IC ₅₀ (μM) Racemate	IC ₅₀ (μM) Enantiomer 1	IC ₅₀ (μM) Enantiomer 2
	0.06	0.28	0.03
	12.7	>>40	6.6
	2.6	>>40	1.3



The *R* enantiomer of B can be crystallized selectively by heating a mixture of B with 1.1 equivalents of *D*-tartaric acid in a mixture of isopropanol and methanol and then
 5 letting the mixture return to room temperature.

Example 9: X = Cl, R = H

Racemic intermediate B (1.5 g), dissolved in 100 mL of boiling isopropanol, was mixed with 0.8 g of *D*-tartaric acid in 100 mL of boiling methanol. The mixture was
 10 allowed to slowly reach room temperature. After standing overnight, the solid was removed by filtration and rinsed with ethyl acetate and hexanes, and allowed to air dry. The dried solid (0.8 g) was then dissolved in a boiling mixture of 50 mL of isopropanol and 50 mL of methanol and allowed to slowly cool to room temperature. After standing overnight, the resulting solid was removed by filtration and rinsed with
 15 ethyl acetate and hexanes, and allowed to air dry. The dried solid was then stirred with saturated sodium bicarbonate for 30 min and extracted with ethyl acetate. The organics were dried (MgSO_4), filtered and evaporated to dryness. The resulting clear oil weighed 345 mg. Chiral purity of >95% was determined by conversion of a portion to the *S*-Mosher amide and examination of the product by $^1\text{HNMR}$.
 20 The enantiomerically pure compounds below were prepared, according to the remaining steps in Figure 4, from material resulting from the procedure described above using both *D*- and *L*-tartaric acid.

The quinazolinone compounds, as well as the known anti-mitotic agent paclitaxel, caused a shift in the population of cells from a G0/G1 cell cycle stage (2n DNA content) to a G2/M cell cycle stage (4n DNA content). Other compounds of this class were found to have similar effects.

5. Monopolar Spindle Formation following Application of a Quinazolinone KSP Inhibitor

To determine the nature of the G2/M accumulation, human tumor cell lines Skov-3 (ovarian), HeLa (cervical), and A549 (lung) were plated in 96-well plates at densities of 4,000 cells per well (SKOV-3 & HeLa) or 8,000 cells per well (A549), allowed to
10 adhere for 24 hours, and treated with various concentrations of the quinazolinone compounds for 24 hours. Cells were fixed in 4% formaldehyde and stained with anti-tubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).

Visual inspection revealed that the quinazolinone compounds caused cell cycle arrest
15 in the prometaphase stage of mitosis. DNA was condensed and spindle formation had initiated, but arrested cells uniformly displayed monopolar spindles, indicating that there was an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

20 Inhibition of Cellular Proliferation in Tumor Cell Lines Treated with Quinazolinone KSP Inhibitors.

Cells were plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate (depending on the cell line) and allowed to adhere/grow for 24 hours. They were then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0 . A tetrazolium-based assay using the reagent
25 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS) (LS> Patent No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQueous One Solution Cell Proliferation Assay) was used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours was compared to

(Sigma P0294), 100 nM KSP motor domain, 50 µg/ml microtubules, 1 mM DTT (Sigma D9779), 5 µM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgCl₂ (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the composition are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 µl of Solution 1. The reaction is started by adding 50 µl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC₅₀ determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{\text{IC}_{50}} \right)^s} + \text{Background}$$

Where y is the observed rate and x the compound concentration.

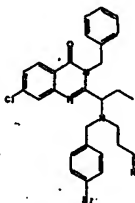
The quinazolinone compounds inhibit growth in a variety of cell lines, including cell lines (MCF-7/ADR-RES, HCT1 5) that express P-glycoprotein (also known as Multi-drug Resistance, or MDR⁺), which conveys resistance to other chemotherapeutic drugs, such as paclitaxel. Therefore, the quinazolinones are anti-mitotics that inhibit cell proliferation, and are not subject to resistance by overexpression of MDR⁺ by drug-resistant tumor lines.

Other compounds of this class were found to inhibit cell proliferation, although GI₅₀ values varied. GI₅₀ values for the quinazolinone compounds tested ranged from 200 nM to greater than the highest concentration tested. By this we mean that although most of the compounds that inhibited KSP activity biochemically did inhibit cell proliferation, for some, at the highest concentration tested (generally about 20 µM), cell growth was inhibited less than 50%. Many of the compounds have GI₅₀

weight x 100% ($\Delta T/\Delta C$) was subtracted from 100% to give the tumor growth inhibition (TGI) for each group.

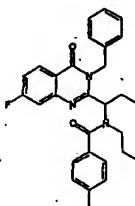
Compounds 1-5 (below) were tested by the above-described method, giving the results summarized below in Tables A - D. Other compounds of the present invention show comparable activities when tested by this method.

Compound 1

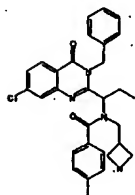


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Compound 2

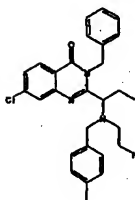


Compound 3



15

Compound 4



20

Compound 5

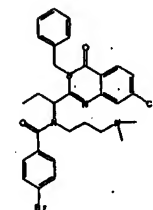


Table B				
SKOV3 tumor xenograft				
	Vehicle	Compound 2	Compound 3	Taxol
Dose & Schedule	Daily x 5	50 mg/kg every 3 days x 4	60 mg/kg daily x 5	20 mg/kg daily x 5
Route	i.v.	i.v.	i.v.	i.p.
# Mice at Start	8	8	8	8
Final Tumor Weight (Mean \pm SEM)	1506.3 \pm 227.1	340.8 \pm 93.0	806.1 \pm 163.8	55.9 \pm 29.4
Tumor Growth Inhibition	—	81.5%	48.3%	99.7%
Mice with Partial Tumor Shrinkage	0	0	0	7
Mean % Tumor Shrinkage	—	—	—	43.5%
Maximum Weight Loss	None	None	2.76%	7.49%
Mortalities	0	0	1	0

Table D			
SKOV3 tumor xenograft			
	Vehicle	Compound 5	Taxol
Dose & Schedule	Daily x 5	25 mg/kg daily x 5	20 mg/kg daily x 5
Route	i.v.	i.v.	i.p.
# Mice at Start	8	8	8
Final Tumor Weight (Mean \pm SEM)	1230.4 \pm 227.3	405.6 \pm 124.8	379.0 \pm 154.0
Tumor Growth Inhibition	—	71.0%	73.0%
Mice with Partial Tumor Shrinkage	0	1	0
Mean % Tumor Shrinkage	—	56.3%	—
Maximum Weight Loss	None	None	8.77%
Mortalities	0	0	0

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto. All patents and publications cited above are hereby incorporated by reference.

R_3 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and R_{15} -NH-;

$R_{3''}$ is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

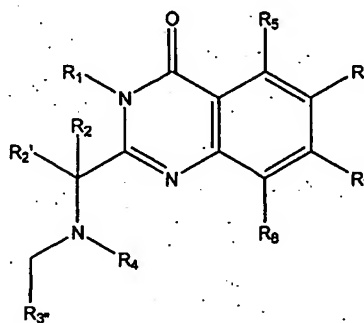
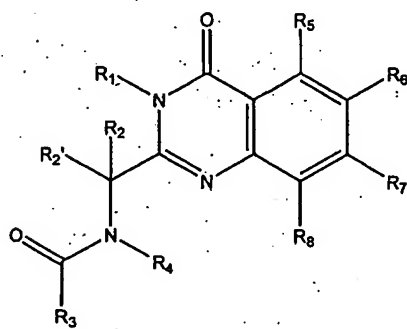
R_4 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and R_{16} -alkylene-;

R_5 , R_6 , R_7 and R_8 are independently chosen from hydrogen, alkyl, alkoxy, halogen, fluoroalkyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, sulfonamidoalkyl, sulfonamidoaryl, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, aryl and heteroaryl;

R_{15} is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; and

R_{16} is chosen from alkoxy, amino, alkylamino, dialkylamino, N-heterocyclyl and substituted N-heterocyclyl, or a pharmaceutically acceptable salt thereof.

2. A method of treating a disorder associated with KSP kinesin activity comprising administering a compound chosen from the group consisting of:

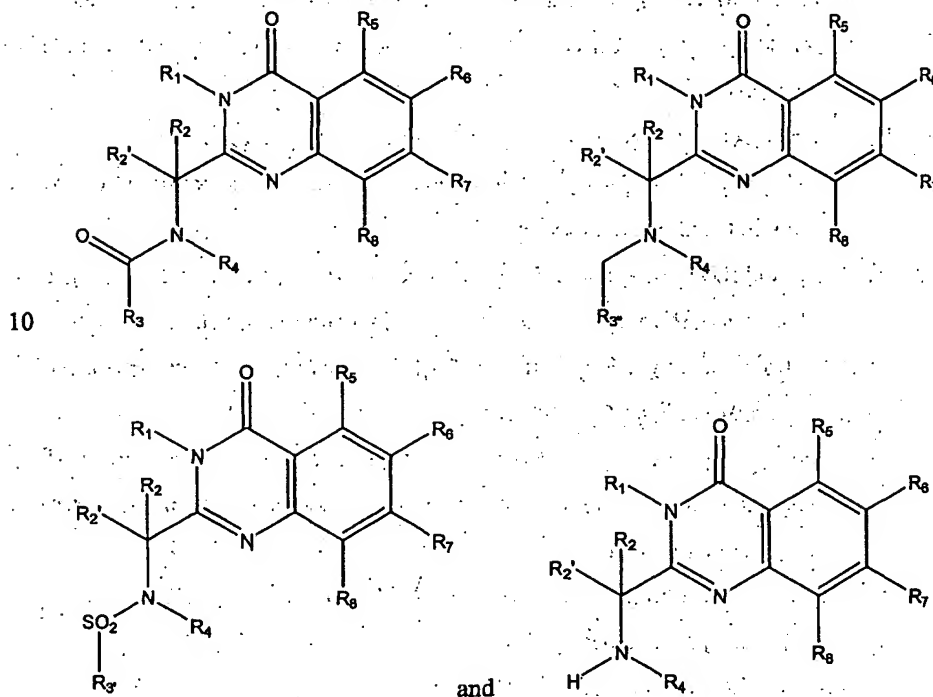


aminocarbonyl, aryl and heteroaryl;

R_{15} is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; and

5 R_{16} is chosen from alkoxy, amino, alkylamino, dialkylamino, N-heterocyclyl and substituted N-heterocyclyl, or a pharmaceutically acceptable salt thereof.

3. A method of inhibiting KSP kinesin comprising contacting KSP kinesin with a compound chosen from the group consisting of:



wherein:

15 R_1 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

R_2 and R_2' are independently chosen from hydrogen, alkyl, oxaalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted

4. A method according to claim 1, 2 or 3 wherein:

R_1 is chosen from hydrogen, alkyl, aryl, substituted alkyl, substituted aryl, heteroaryl, substituted heteroaryl, alkylaryl, substituted alkylaryl and substituted alkylheteroaryl;

5 R_2 is chosen from hydrogen, alkyl and substituted alkyl;

R_2' is hydrogen;

R_3 is chosen from alkyl, substituted alkyl, alkylaryl, heteroaryl, aryl, substituted aryl, substituted heteroaryl, substituted oxaalkylaryl $R_{15}O-$ and $R_{15}NH-$;

10 R_4 is chosen from alkyl, aryl, alkylaryl, alkylheteroaryl, substituted alkyl, substituted aryl, and R_{16} -alkylene-;

R_5 is hydrogen;

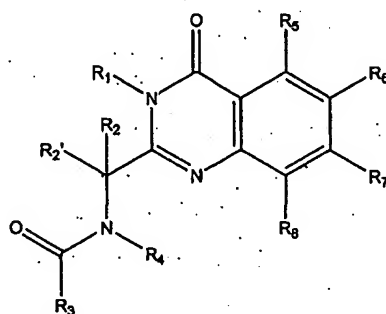
R_6 , R_7 and R_8 are independently chosen from hydrogen, halogen, methyl, cyano and trifluoromethyl;

R_{15} is chosen from alkyl, aryl and substituted aryl;

15 R_{16} is chosen from alkoxy, amino, alkylamino, dialkylamino and N-heterocyclyl.

5. A method according to claim 1, 2 or 3 wherein: R_2 is chosen from hydrogen, alkyl and substituted alkyl; R_2' is hydrogen; and the stereogenic center to which R_2 and R_2' are attached is of the R configuration.

6. A method according to claim 1, 2 or 3 comprising administering a compound of the formula:



or a pharmaceutically acceptable salt thereof.

indolyl, methylpyridinyl, quinolinyl, picolinyl, pyrazolyl, and imidazolyl.

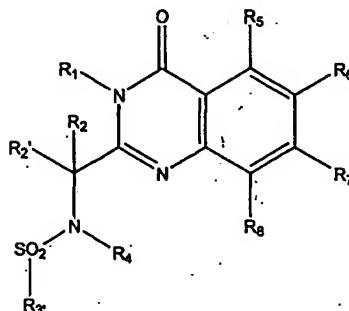
13. A method according to claim 6 wherein R_3 is R_{15} -NH- and R_{15} is chosen from lower alkyl; cyclohexyl; phenyl; and phenyl substituted with halo, lower alkyl, loweralkoxy, or lower alkylthio.

5 14. A method according to claim 13 wherein R_{15} is chosen from isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, trifluoromethylphenyl and methylthiophenyl.

15 15. A method according to claim 6 wherein R_4 is chosen from lower alkyl, substituted lower alkyl, cyclohexyl; phenyl substituted with hydroxy, lower alkoxy or
10 lower alkyl; benzyl; heteroarylmethyl; heteroarylethyl; heteroarylpropyl and R_{16} -alkylene-, wherein R_{16} is amino, lower alkylamino, di(lower alkyl)amino, lower alkoxy, or N-heterocyclyl.

16. A method according to claim 15 wherein R_4 is chosen from methyl,
ethyl, propyl, butyl, cyclohexyl, carboxyethyl, carboxymethyl, methoxyethyl,
15 hydroxyethyl, hydroxypropyl, dimethylaminoethyl, dimethylaminopropyl, diethylaminoethyl, diethylaminopropyl, aminopropyl, methylaminopropyl, 2,2-dimethyl-3-(dimethylamino)propyl, 1-cyclohexyl-4-(diethylamino)butyl, aminoethyl, aminobutyl, aminopentyl, aminohexyl, aminoethoxyethyl, isopropylaminopropyl, diisopropylaminoethyl, 1-methyl-4-(diethylamino)butyl, (t-Boc)aminopropyl,
20 hydroxyphenyl, benzyl, methoxyphenyl, methylmethoxyphenyl, dimethylphenyl, tolyl, ethylphenyl, (oxopyrrolidinyl)propyl, (methoxycarbonyl)ethyl, benzylpiperidinyl, pyridinylethyl, pyridinylmethyl, morpholinylethyl, morpholinylpropyl, piperidinyl, azetidinylmethyl, azetidinypropyl, pyrrolidinylethyl, pyrrolidinylpropyl, piperidinylmethyl, piperidinylethyl, imidazolylpropyl, imidazolylethyl,
25 (ethylpyrrolidinyl)methyl, (methylpyrrolidinyl)ethyl, (methylpiperidinyl)propyl, (methylpiperazinyl)propyl, furanylmethyl and indolylethyl.

20. A method according to claim 1, 2 or 3 comprising administering a compound of formula:



or a pharmaceutically acceptable salt thereof.

21. A method according to claim 20 wherein:

R₁ is chosen from hydrogen, lower alkyl, substituted lower alkyl, benzyl, substituted benzyl, phenyl, naphthyl and substituted phenyl;

R₂ is chosen from hydrogen, lower alkyl and substituted lower alkyl and R₂' is hydrogen;

R₃ is chosen from C₁-C₁₃ alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, nitro, methylenedioxy, or trifluoromethyl; biphenyl, benzyl and heteroaryl; and

R₄ is chosen from lower alkyl, substituted lower alkyl, cyclohexyl; phenyl substituted with hydroxy, lower alkoxy or lower alkyl; benzyl; heteroarylmethyl;

heteroarylethyl; heteroarylpropyl and R₁₆-alkylene, wherein

R₁₆ is amino, (lower alkyl)amino, di(lower alkyl)amino, lower alkoxy, or N-heterocyclyl.

22. A method according to claim 20 wherein

R₁ is chosen from lower alkyl, benzyl, substituted benzyl and substituted phenyl;

R₂ is hydrogen or lower alkyl;

R₂' is hydrogen;

R₃ is chosen from substituted phenyl and naphthyl;

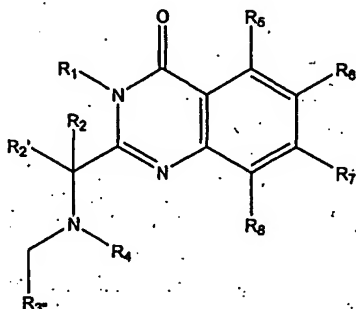
R₄ is R₁₆-alkylene-, hydroxy lower alkyl or carboxy lower alkyl;

R₆ and R₇ are chosen from hydrogen and halo;

R_5 and R_8 are hydrogen; and

R_{16} is chosen from di(lower alkylamino), (lower alkyl)amino, amino, pyrrolidinyl, piperidinyl, imidazolyl and morpholinyl.

26. A method according to claim 1, 2 or 3 comprising administering a
5 compound of formula:



27. A method according to claim 26 wherein:

R_1 is chosen from hydrogen, lower alkyl, substituted lower alkyl, benzyl, substituted benzyl, phenyl, naphthyl and substituted phenyl;

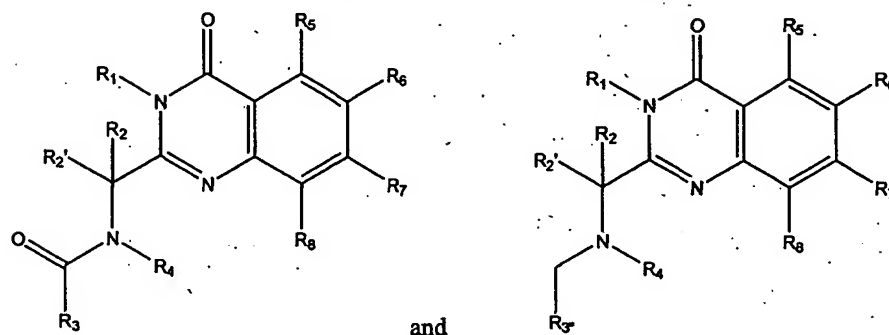
- 10 R_2 is chosen from hydrogen, lower alkyl and substituted lower alkyl and R_2' is hydrogen;

$R_{3'}$ is chosen from C_1 - C_{13} alkyl; substituted lower alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, nitro, methylenedioxy, or trifluoromethyl; biphenyl; benzyl and heterocyclyl; and

- 15 R_4 is chosen from lower alkyl, substituted lower alkyl; cyclohexyl; phenyl substituted with hydroxy, lower alkoxy or lower alkyl; benzyl; substituted benzyl; heterocyclyl; heteroaryl methyl; heteroarylethyl; heteroarylpropyl and R_{16} -alkylene, wherein

R_{16} is di(lower alkyl)amino, (lower alkyl)amino, amino, lower alkoxy, or N-
20 heterocyclyl.

31. A compound chosen from the group consisting of:



5 wherein:

R_1 is chosen from hydrogen, alkyl, alkylaryl, alkylheteroaryl, substituted alkyl, substituted alkylaryl, and substituted alkylheteroaryl;

R_2 and R_2' are independently chosen from hydrogen, alkyl, oxaalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; or R_2 and R_2' taken together form a 3- to 7-membered ring;

R_3 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, oxaalkyl, oxaalkylaryl, substituted oxaalkylaryl, oxaalkylheteroaryl, substituted oxaalkylheteroaryl, $R_{15}O-$ and $R_{15}NH-$;

R_3' is chosen from alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

R_4 is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, and R_{16} -alkylene-;

R_5 , R_6 , R_7 and R_8 are independently chosen from hydrogen, alkyl, alkoxy, halogen, fluoroalkyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, sulfonamidoalkyl, sulfonamidoaryl, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, aryl and heteroaryl;

R_{15} is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl,

R₅, R₆, R₇ and R₈ are independently chosen from hydrogen, alkyl, alkoxy, halogen, fluoroalkyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, sulfonamidoalkyl, sulfonamidoaryl, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, aryl and heteroaryl;

5 R₁₅ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; and

R₁₆ is chosen from alkoxy, amino, alkylamino, dialkylamino, N-heterocyclyl and substituted N-heterocyclyl,

10 or a pharmaceutically acceptable salt thereof.

33. A compound or salt according to claim 31 or 32 wherein:

R₁ is chosen from hydrogen, alkyl, aryl, substituted alkyl, substituted aryl, heteroaryl, substituted heteroaryl, alkylaryl, alkylheteroaryl and substituted alkylaryl;

15 R₂ is chosen from hydrogen, alkyl and substituted alkyl; R₂' is hydrogen; and the stereogenic center to which R₂ and R₂' are attached is of the R configuration.

R₃ is chosen from alkyl, aryl, alkylaryl, heteroaryl, substituted aryl, substituted alkyl, substituted heteroaryl, oxaalkylaryl, substituted oxaalkylaryl, R₁₅O- and R₁₅-NH-;

20 R₄ is chosen from alkyl, aryl, alkylaryl, alkylheteroaryl, substituted alkyl, substituted aryl, and R₁₆-alkylene-;

R₅ is hydrogen;

R₆, R₇ and R₈ are independently chosen from hydrogen, halogen, methyl and trifluoromethyl;

R₁₅ is chosen from alkyl, aryl and substituted aryl; and

25 R₁₆ is chosen from alkoxy, amino, alkylamino, dialkylamino and N-heterocyclyl.

41. A compound or salt according to claim 40 wherein R_3 is chosen from halophenyl, dihalophenyl, cyanophenyl, halo(trifluoromethyl)phenyl, chlorophenoxymethyl, methoxyphenyl, carboxyphenyl, methylphenyl, ethylphenyl, tolyl, biphenyl, methylenedioxyphenyl, methylsulfonylphenyl, methoxychlorophenyl; methylhalophenyl, trifluoromethylphenyl, butylphenyl, pentylphenyl, methylnitrophenyl, dimethoxyphenyl, phenylvinyl, nitrochlorophenyl, nitrophenyl, dinitrophenyl, bis(trifluoromethyl)phenyl,
42. A compound or salt according to claim 37 wherein R_3 is R_{15} -NH- where R_{15} is chosen from isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, trifluoromethylphenyl and methylthiophenyl.
43. A compound or salt according to claim 34, 37 or 39 wherein R_4 is chosen from lower alkyl, substituted lower alkyl, and R_{16} -alkylene-, wherein R_{16} is amino, lower alkylamino, di(lower alkyl)amino, lower alkoxy, or N-heterocyclyl.
44. A compound or salt according to claim 34, 37 or 39 wherein R_5 , R_6 , R_7 and R_8 are chosen from hydrogen, halo, lower alkyl, substituted lower alkyl, lower alkoxy and cyano.
45. A compound or salt according to claim 44 wherein R_5 , R_6 and R_8 are hydrogen.
46. A compound or salt according to claim 45 wherein R_7 is halo.

50. The compound or salt according to claim 47 wherein:

R₁ is benzyl;

R₂ is i-propyl;

R₃ is p-fluorophenyl;

5 R₄ is 3-amino-n-propyl;

R₅ is hydrogen;

R₆ is hydrogen;

R₇ is chloro; and

R₈ is hydrogen.

10

51. The compound or salt according to claim 47 wherein:

R₁ is benzyl;

R₂ is i-propyl;

R₃ is 3-fluoro-4-methylphenyl;

15 R₄ is 3-amino-n-propyl;

R₅ is hydrogen;

R₆ is hydrogen;

R₇ is chloro; and

R₈ is hydrogen.

20

52. The compound or salt according to claim 47 wherein:

R₁ is benzyl;

R₂ is i-propyl;

R₃ is p-methylphenyl;

25 R₄ is 3-amino-n-propyl;

R₅ is hydrogen;

R₆ is hydrogen;

R₇ is chloro; and

R₈ is hydrogen.

30

56. A compound according to claim 55 wherein:

R_1 is chosen from lower alkyl, benzyl, substituted benzyl and substituted phenyl;

R_2 is hydrogen or lower alkyl;

R_2' is hydrogen;

5 R_3 is chosen from substituted phenyl and naphthyl;

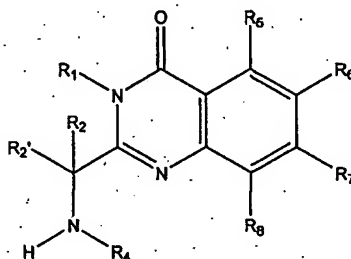
R_4 is R_{16} -alkylene-, hydroxy(lower alkyl) or carboxy (lower alkyl);

R_7 is hydrogen, fluoro, chloro or methyl;

R_5 , R_6 and R_8 are hydrogen;

R_{16} is chosen from di(lower alkyl)amino, (lower alkyl)amino, amino, pyrrolidinyl and
10 piperidinyl.

57. A compound according to claim 32 of formula:



or a pharmaceutically acceptable salt thereof.

58. A compound or salt according to claim 57 wherein:

15 R_1 is chosen from hydrogen, lower alkyl, substituted lower alkyl, benzyl, substituted benzyl, phenyl, naphthyl and substituted phenyl;

R_2 is chosen from hydrogen, lower alkyl and substituted lower alkyl and R_2' is hydrogen; and

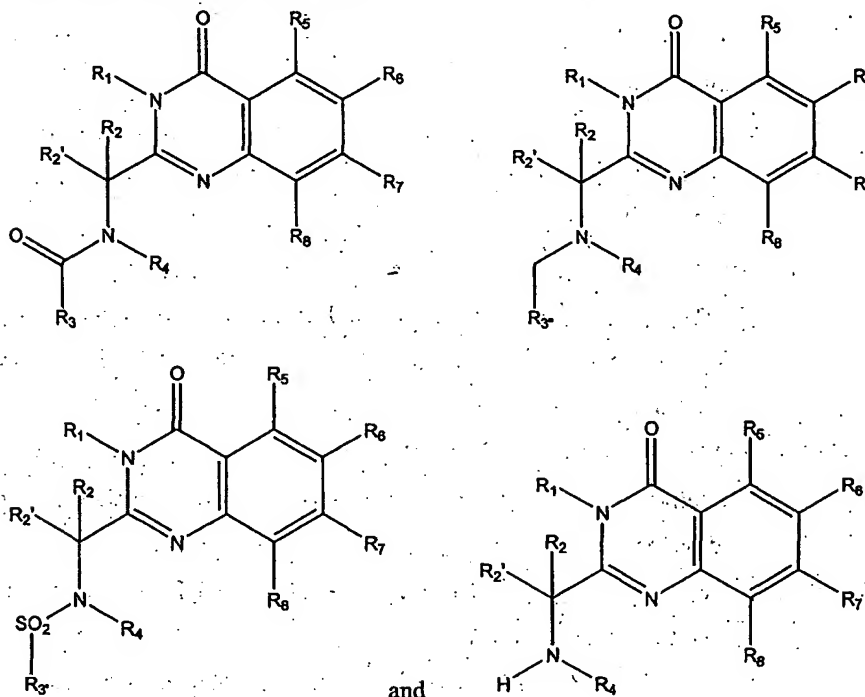
R_4 is chosen from lower alkyl, cyclohexyl; phenyl substituted with hydroxy, lower
20 alkoxy or lower alkyl; benzyl; heteroaryl methyl; heteroarylethyl;
heteroarylpropyl and R_{16} -alkylene, wherein R_{16} is di(lower alkyl)amino,
(lower alkyl)amino, amino, lower alkoxy, or N-heterocyclyl.

62. A compound according to claim 31 wherein said compound is of a formula as defined in Figure 3.

63. A method of screening for KSP kinesin modulators comprising:

(a) combining a kinesin, a candidate bioactive agent and a compound

5 chosen from the group consisting of:



and

wherein:

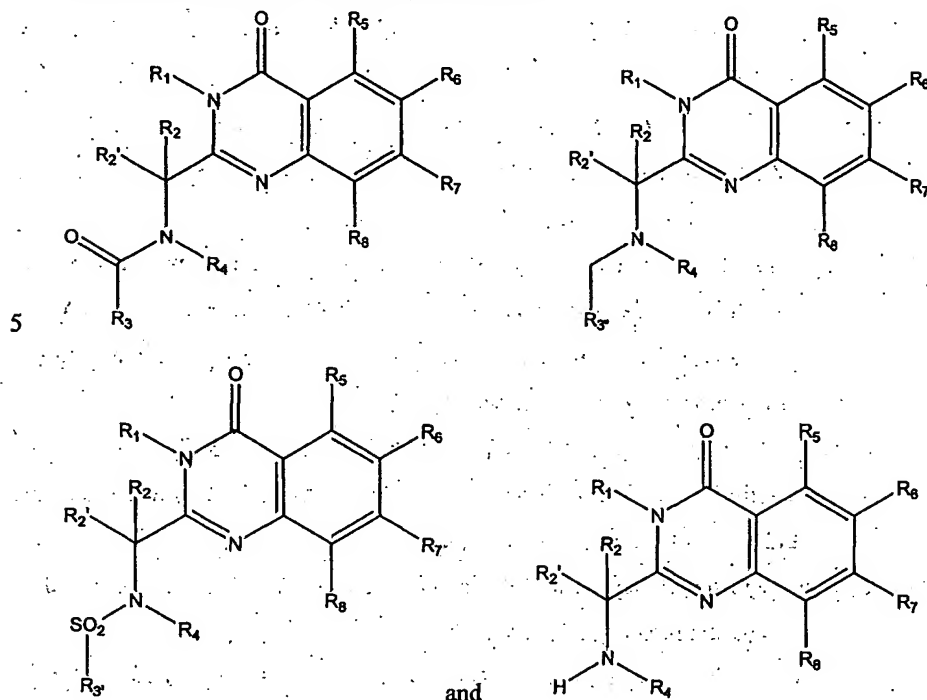
10 R₁ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

R₂ and R_{2'} are independently chosen from hydrogen, alkyl, oxaalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; or R₂ and R_{2'} taken together form a 3- to 7-membered ring;

15 R₃ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl,

64. A method of screening for compounds that bind to KSP kinesin comprising:

(a) combining a kinesin, a candidate bioactive agent and a labeled compound chosen from the group consisting of:



wherein:

R₁ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl;

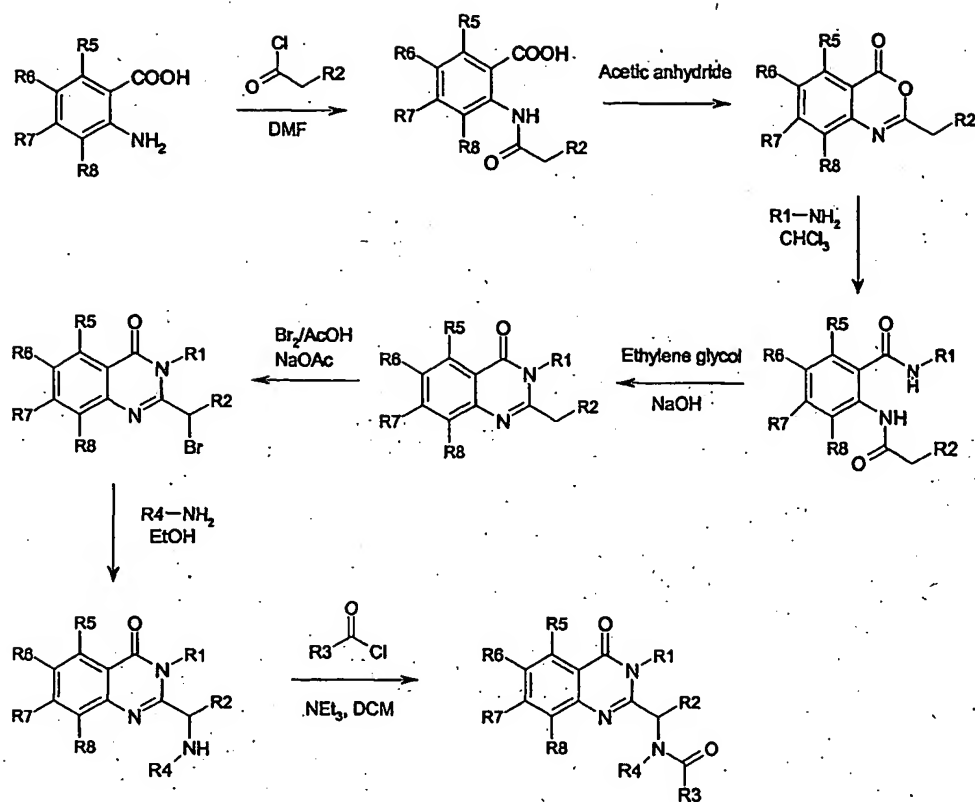
R₂ and R₂' are independently chosen from hydrogen, alkyl, oxaalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, and substituted alkylheteroaryl; or R₂ and R₂' taken together form a 3- to 7-membered ring;

R₃ is chosen from hydrogen, alkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, substituted alkyl, substituted aryl, substituted alkylaryl, substituted heteroaryl, substituted alkylheteroaryl, oxaalkyl, oxaalkylaryl, substituted oxaalkylaryl, oxaalkylheteroaryl, substituted oxaalkylheteroaryl, R₁₅O- and R₁₅NH-;

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Figure 1

5



R ₁	R ₂	R ₃	R ₄	R ₆	R ₈	R ₇	R ₉	Activity
						Cl X ₇		%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3.

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

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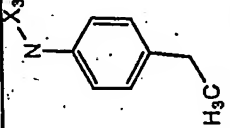
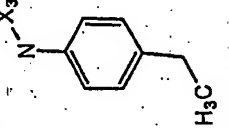
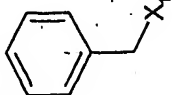
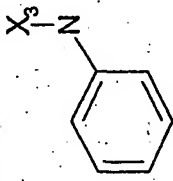
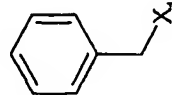
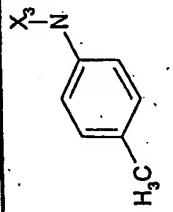
R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
	$\text{X}_2 - \text{CH}_3$		$\text{H}_3\text{C} - \text{N}(\text{CH}_3) - \text{CH}_2 - \text{CH}_2 - \text{X}_4$			$\text{X}_7 - \text{Cl}$		%I > 35% @ 40 uM
	$\text{X}_2 - \text{CH}_3$		$\text{H}_3\text{C} - \text{N}(\text{CH}_3) - \text{CH}_2 - \text{CH}_2 - \text{X}_4$			$\text{X}_7 - \text{Cl}$		%I > 35% @ 40 uM
	$\text{H}_3\text{C} - \text{CH}_2 - \text{X}_2$		$\text{X}_4 - \text{CH}_2 - \text{CH}_2 - \text{N}(\text{CH}_3)_2$					%I > 35% @ 40 uM
	$\text{H}_3\text{C} - \text{CH}_2 - \text{X}_2$		$\text{X}_4 - \text{CH}_2 - \text{CH}_2 - \text{N}(\text{CH}_3)_2$					%I > 35% @ 40 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

27/90

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

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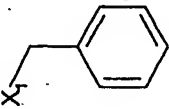

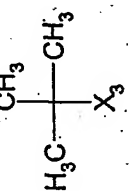
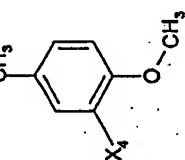
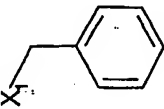

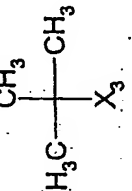
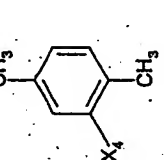
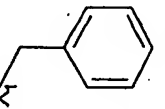
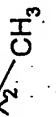
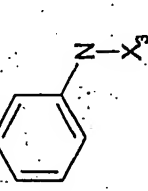
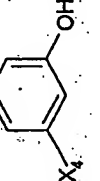
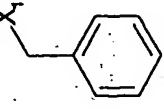


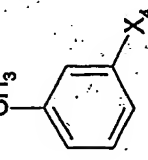
R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM
								%I > 35% @ 40 uM

Figure 3 (continued)

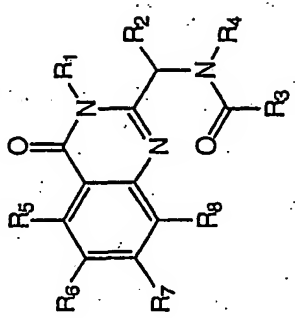
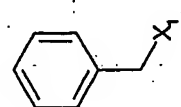
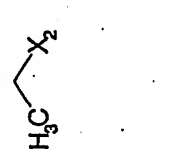
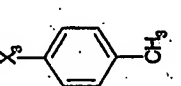
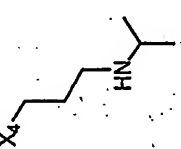

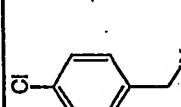
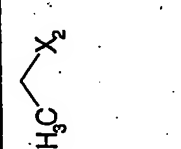
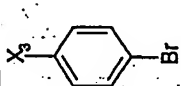
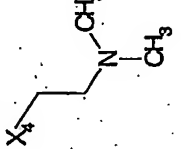
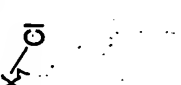
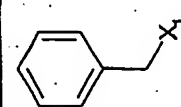
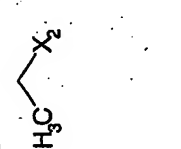
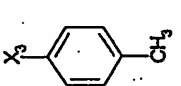


									
R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity	
								IC ₅₀ < 100 nM	
								IC ₅₀ < 100 nM	
								IC ₅₀ < 100 nM	

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

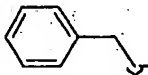
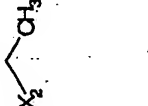

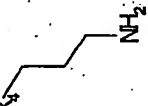

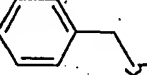
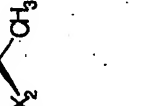
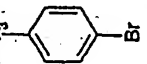
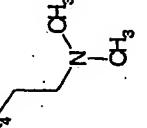
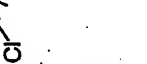
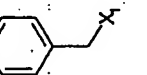
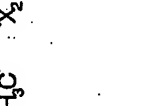
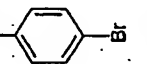
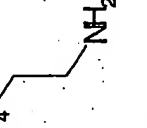
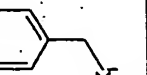

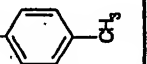
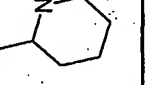

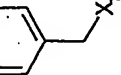

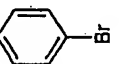
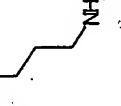

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM
								IC50 < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 μ M
								IC50 = 100 nM-1 μ M
								IC50 = 100 nM-1 μ M
								IC50 = 100 nM-1 μ M
								IC50 = 100 nM-1 μ M

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM
								IC50 = 100 nM-1 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	Activity
							IC50 = 1 uM-10 uM
							IC50 = 1 uM-10 uM
							IC50 = 1 uM-10 uM
							IC50 = 1 uM-10 uM
							IC50 = 1 uM-10 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₆	R ₈	Activity
						IC50 = 1 uM-10 uM
						IC50 = 1 uM-10 uM
						IC50 = 1 uM-10 uM
						IC50 = 1 uM-10 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC ₅₀ = 1 μ M-10 μ M
								IC ₅₀ = 1 μ M-10 μ M
								IC ₅₀ = 1 μ M-10 μ M
								IC ₅₀ = 1 μ M-10 μ M
								IC ₅₀ = 1 μ M-10 μ M

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 1 uM-10 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM

Figure 3 (continued)

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R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM

Figure 3 (continued)

R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM
								IC50 = 10 uM-50 uM

Figure 3 (continued)

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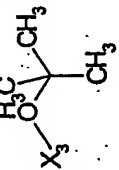
R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								IC ₅₀ = 10 μM-50 μM

Figure 3 (continued)

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R ₁	R ₂	R ₃ ^{''}	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃ "	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM

Figure 3 (continued)

R ₁	R ₂	R ₃ ^{''}	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM

Figure 3 (continued)

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R ₁	R ₂	R ₃ ^{''}	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM

Figure 3 (continued)

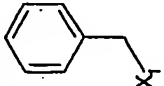
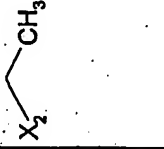
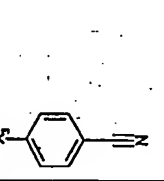
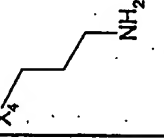
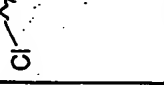
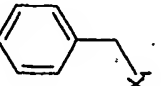
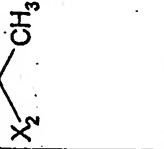
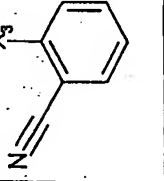
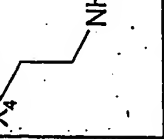

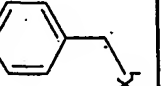
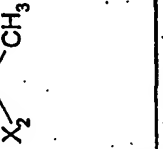
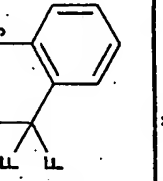
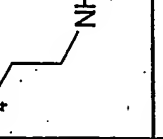

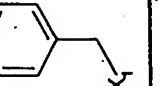
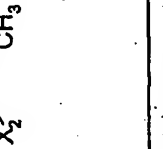
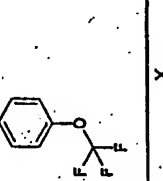
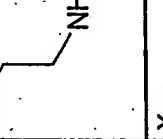
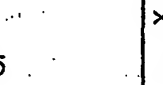
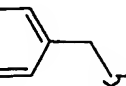
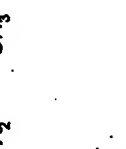
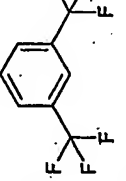
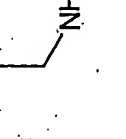
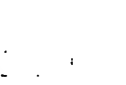
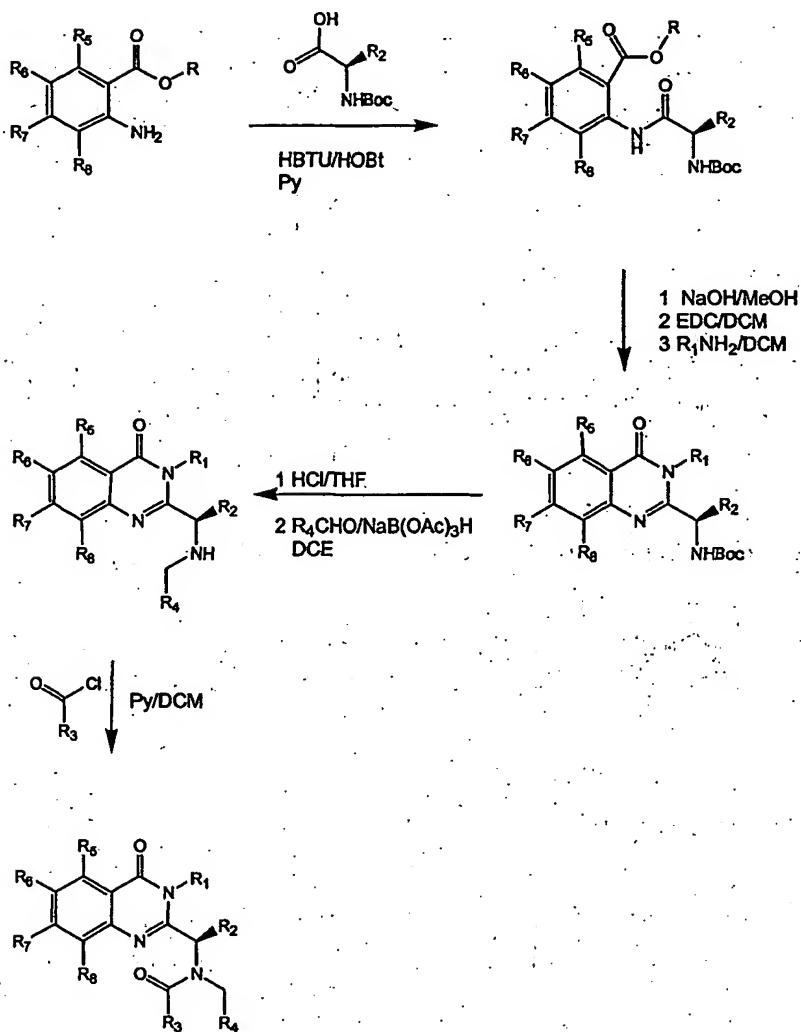
R ₁	R ₂	R ₃ "	R ₄	R ₅	R ₆	R ₇	R ₈	Activity
								KI < 100 nM
								KI < 100 nM
								KI < 100 nM
								KI = 100 nM-1 uM
								KI = 100 nM-1 uM

Figure 3 (continued)

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Figure 4: Asymmetric Synthesis



INTERNATIONAL SEARCH REPORT

International Application No.

PC1/US 01/13901

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D239/91 A61K31/517 A61P37/02 C07D239/90 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.K. DEBNATH:-- "STRUCTURE BASED IDENTIFICATION OF SMALL MOLECULE ANTIVIRAL COMPOUNDS" JOURNAL OF MEDICINAL CHEMISTRY., vol. 42, no. 17, 26 August 1999 (1999-08-26), pages 3203-9, XP002159846 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US ISSN: 0022-2623 page 3203 -page 3206 -- -/-	1, 31, 32, 49, 57, 58, 63

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

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O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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A document member of the same patent family

Date of the actual completion of the international search

23 October 2001

Date of mailing of the international search report

31/10/2001

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International Application No
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